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Abstract

The presence of more than 2 cm (height) of uterine fluid during estrus seems to be a predictor of susceptibility to post-breeding endometritis.

1. Introduction

The mare's uterus is exposed to a variety of contaminants and infectious agents at the time of breeding. Antigenic material in semen will induce some degree of endometrial inflammatory response [1,2]. A transient inflammatory reaction seems to be a normal response to breeding and is thought to help rid the uterus of contaminants [3]. It is widely accepted that impaired uterine clearance is a major cause of chronic recurrent endometritis. Resistant mares have intact uterine defense mechanisms, and they are able to overcome intrauterine contamination and mitigate the inflammatory response within a short period of time [4]. Susceptible mares are prone to chronic, persistent post-breeding endometritis, because they have impaired uterine defense mechanisms and are unable to clear their uteri of luminal contaminants and inflammatory products [4].

Historically, mares have been classified as being susceptible to persistent, post-mating endometritis because of advanced age [5], increased parity [5], presence of chronic inflammatory changes within the endometrium [6], presence of intra-uterine fluid at diestrus [2], failure to clear an intra-uterine bacterial challenge, [8], or their ability to clear intra-uterine radiocolloid within 2 h. However, these measures of uterine health or function are not always practical in a clinical setting. Identification of susceptible mares on breeding farms is often made "after the fact". The presence of intra-uterine fluid is often detected after breeding or ovulation, when the opportunity for therapeutic intervention may be compromised. This delay in intervention often results in lowered pregnancy rates, and it has a substantial economic impact on the equine breeding industry. The purpose of this study was to determine if mares with factors associated with susceptibility to persistent mating-induced endometritis could be identified in estrus. The ability of clinicians to more accurately predict the susceptibility of mares to delayed uterine clearance before breeding should facilitate timely uterine treatment and help to reduce chronic post-breeding endometritis.

2. Materials and Methods

A total of 27 mares were studied over 2 yr, 15 mares in the first year and 12 mares in the second year. Mares were categorized as resistant (aged 3 - 20 yr, n = 14) or susceptible (aged 13 - 24 yr, n = 13). Classification was based on reproductive history, breeding soundness, reproductive tract examination (including evaluation of endometrial biopsies [10] at the start of the study), and clinical evaluation of uterine function during consecutive estrus periods. Evaluation of uterine function included (1) presence of free uterine fluid during diestrus [7], (2) presence of uterine fluid 72 h after insemination [11], (3) presence of uterine fluid 96 h after bacterial challenge with Streptococcus zooepidemicus, [8], (4) positive uterine culture 96 h after bacterial challenge with Streptococcus zooepidemicus [8], and (5) by scintigraphy after intrauterine inoculation with a radiocolloid [9]. Mares that were positive in three or more clinical evaluation criteria were classified as susceptible [11]. Presence or absence of luminal uterine fluid, maximum height of luminal uterine fluid, location of fluid within the uterus, and stage of the mare's estrous cycle were recorded at each examination.
3. Analysis of Data
Clinical evaluations of uterine function were evaluated as dichotomous ("yes" or "no") data in 2 x 2 tables to examine the strength of their agreement using the \( \kappa \) statistic [12]. \( \kappa \) represents the proportion of agreement beyond that occurring by chance that was actually achieved. The following qualitative terms of agreement were assigned to \( \kappa \) values: "slight" (0.00-0.2), "fair" (0.2-0.4), "moderate" (0.4-0.6), "substantial" (0.6-0.8), and "almost perfect" (0.8-1.0) [13]. Agreement among evaluation techniques was examined for all mares and within groups of mares classified as susceptible or resistant to post-breeding endometritis.

4. Results
During the course of the study, it was noted that a number of mares accumulated varying amounts of luminal uterine fluid during estrus. All 13 mares classified as susceptible accumulated fluid during estrus, and 6 of 14 resistant mares also had some degree of fluid accumulation during estrus. The highest level of agreement for fluid during estrus and the five criteria used to determine susceptibility to post-breeding endometritis was \( \kappa = 0.33 \) (fair), associated with fluid accumulation 72 h after insemination. The degree of agreement with estrous fluid and the presence of fluid during diestrus was only fair (\( \kappa = 0.25 \)). The highest level of agreement for fluid during estrus and any of the five criteria used to determine susceptibility to post-breeding endometritis was \( \kappa = 0.33 \) (fair) and was associated with fluid accumulation 72 h after insemination. However, when estral fluid > 2 cm in height was evaluated, much stronger agreements were observed (Table 1). Twelve of the 13 mares classified as being susceptible to post-breeding endometritis had estrous fluid > 2 cm in height, whereas only 2 of 14 resistant mares demonstrated this characteristic.

<table>
<thead>
<tr>
<th>Table 1. Measures of Agreement Between the Presence of Fluid &gt; 2 cm in Height During Estrus, Fluid During Diestrus, Clearance of &lt; 15% Radiocolloid Measured by Scintigraphy, and Factors Used to Classify Mares as Susceptible to Post-breeding Endometritis (n = 27)</th>
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<tbody>
<tr>
<td>Factor</td>
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<tr>
<td>Overall Agreement</td>
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<td>-----------------</td>
</tr>
<tr>
<td>Diestral fluid</td>
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<tr>
<td>Fluid 72 h after insemination</td>
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<tr>
<td>Fluid 96 h after inoculation*</td>
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<tr>
<td>Positive culture 96 h after inoculation *</td>
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<td>&lt; 15% clearance of radiocolloid</td>
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* Intra-uterine inoculation of a 30-ml suspension containing 3 x 10^8 CFU of *Streptococcus zooepidemicus* in the presence of an estral follicle > 35 mm in diameter.

Surprisingly, the amount of agreement between the factor most widely regarded as an indicator of susceptibility to endometritis (i.e., the presence of uterine luminal fluid during diestrus) and other factors considered to be indicators was not very strong (Table 1). The amount of agreement between scintigraphy (clearance of < 15% of radiocolloid 2 h after inoculation) and other factors was also disappointingly low (Table 1). In addition, the criteria reported to classify mares as resistant to post-breeding endometritis using scintigraphy (i.e., > 50% clearance of radiocolloid 2 h after inoculation) also had poor agreement, regardless of whether or not the mares were classified as resistant (overall agreement = 17/27; \( \kappa = 0.27 \)). Of the factors used to classify mares as susceptible, fluid during diestrus (\( \kappa = 0.71 \); overall agreement = 23/27) and fluid 72 h after insemination (\( \kappa = 0.70 \); overall agreement = 23/27) demonstrated the highest levels of agreement. However, when the presence of > 2 cm of fluid during estrus is examined as a predictor of mares classified as susceptible to post-breeding endometritis (n = 13), the level of agreement beyond that achieved by chance was the highest in the study (\( \kappa = 0.80 \); overall agreement = 24/27).

5. Discussion
These data suggest that clinicians can predict if mares are susceptible to delayed uterine clearance based on the quantity of intra-uterine fluid present during estrus. In the current study, for a mare to be classified as susceptible to post-breeding endometritis, she had to exhibit at least three factors found in previous studies to be associated with delayed uterine clearance.
It is most interesting that intra-uterine fluid measuring > 2 cm in height during estrus had a higher strength of agreement with mares classified as being susceptible than did any of the individual factors used to classify the mares. Of the factors used to classify mares as susceptible, fluid during diestrus and fluid 72 h after insemination had the strongest agreement with mares in that classification.

Some practitioners feel that mares with fluid during estrus should be viewed with suspicion, whereas others feel that it is inconsequential. Investigators have reported that mares with uterine fluid before mating had reduced pregnancy rates compared with mares that did not have intra-uterine fluid before breeding [14,15]. Other investigators have reported reduced pregnancy rates associated with estral fluid in some studies but not in others [10]. Data from the current study indicate that the amount of fluid present during estrus is more indicative of whether a mare has an increased susceptibility to mating-induced endometritis than does the presence of fluid during estrus alone. These clinical findings are supported by results of an in vitro companion study performed on uterine muscle strips obtained from the same mares. In that study [11], there was a significant correlation between maximal active tension and the presence of uterine fluid both during estrus and 72 h after insemination. Myometrium harvested from mares that accumulated larger volumes of fluid during estrus generated less tension in vitro.

In summary, it seems that the presence of estral uterine fluid > 2 cm in height is a good indicator that mares are susceptible to mating-induced endometritis. This information will be particularly useful to broodmare practitioners when they are presented with mares with an unknown or nebulous reproductive history. The ability to identify susceptible mares before breeding will facilitate timely therapeutic intervention and help prevent persistent post-breeding endometritis in susceptible mares.

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References


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